Services Provided in Support of the Planetary Quarantine Requirements of the National Aeronautics and Space Administration under Contract W-13,062.

Report No. 37
January - March 1972

Applied Microbiology and Planetary Quarantine Section
Phoenix Laboratories
Ecological Investigations Program
Center for Disease Control
Public Health Service
U.S. Department of Health, Education, and Welfare
Phoenix, Arizona

Contributors:

Biophysics Unit	Experimental Microbiology Unit	Spacecraft Bioassay Unit
N. Petersen	W. Bond	J. Puleo
J. Marshall	L. Carson	G. Oxborrow
D. Collins	M. Korber	N. Fields
		C. Herring

Report submitted by:

Martin S. Favero, Ph.D., Chief
Applied Microbiology and
Planetary Quarantine Section

Report reviewed and forwarded by:

James E. Maynard, M.D., Chief
Ecological Investigations Program
Phoenix Laboratories

1. As mentioned in earlier reports (Q.R. #35 and #36), a large scale study was initiated at Cape Kennedy to determine the thermal resistance of naturally occurring airborne spores. The Manned Spacecraft Operations Building (MSOB) was selected as the test area. Basically the experiments consist of exposing 3" x 72" teflon ribbons to fallout contamination in the test area for 1 week. The ribbons are rolled up, inserted in sterile jars and placed in an oven at 125 C for various times. After heating, sterile Trypticase Soy Broth supplemented with yeast extract and starch is added and the units are incubated for 28 days at 32 C. D_{125C} values are calculated by the method described by Pflug and Schmidt (fractional-replicate-unit-negative-MPN: Q.R. #26). To date, 18 tests have been performed and these are described in part 4 of this report. One problem with the study has been the relatively low level of spores (ca 100) collected on each ribbon. A spore concentration of 10^4-10^5/ribbon would be ideal since it would provide for a population reduction of several logs. It appears doubtful that spore concentrations of this magnitude can be achieved in spacecraft assembly areas currently in use. Consequently an auxiliary study was designed in which sieve-processed vacuum cleaner dust could be aerosolized and subsequently deposited on teflon ribbons. A chamber was designed and built in Phoenix for this purpose.

   The basic design criteria required spore laden dust to be deposited on 32 teflon ribbons (each 3" x 72") simultaneously to provide a uniform concentration of approximately 5 x 10^4 spores per ribbon. To meet these criteria a scaled-up version of a system for aerosolization and deposition of dust described earlier (Q.R. #22) was designed.

   The chamber is shown in Figure 1 and consisted essentially of a plywood box 108" x 84" x 51". Attached to the front of the chamber was a smaller box (7" x 7" x 7") with one transparent wall, a 1 1/2" inlet at the top for a high velocity air source and a 1 1/2" outlet through a plastic pipe into the larger chamber. The 1 1/2" (I.D.) pipe terminates directly beneath an 11" fan. Two 3" high openings at the bottom of either side of the chamber permitted insertion of two 48" x 77" x 1/4" panels into the chamber. These panels were equipped with spring clips to hold the teflon strips in place in a horizontal position at the bottom of the chamber. Three inches above the panels, guides were built into the sides of the chamber to support two 84" x 54" x 1/4" covers that served to prevent dust from reaching the teflon ribbons during the aerosolization and mixing stages but could be removed to permit deposition of the airborne dust onto the ribbons.

   The procedure for depositing dust on the teflon ribbons began by clipping 32 sterile ribbons to the panels in a laminar downflow clean room. The panels had been previously wiped clean with 95% ethanol. Rodac assays of the panels showed residual contamination levels to be negligible. The covers were similarly cleaned and inserted into the chamber. The ribbon panels were then inserted under the covers. Ten grams of mixed, sieve-processed dust were placed on the floor of the small aerosolization

box. The fan in the chamber was started and a stream of high-velocity air was directed into the aerosolization box for 10 seconds. The dust was completely aerosolized and driven through the pipe into the fan which thoroughly dispersed the dust throughout the chamber air. After the 10 second aerosolization period, the chamber contents were mixed for 10 seconds and the fan was turned off. After a 10 second calming period, the covers were removed from the chamber and all openings into the chamber were quickly closed and taped. The airborne dust was then permitted to settle onto the teflon ribbons for a period of 54 hours. At that time the ribbon panels were slowly withdrawn from the chamber and the ribbons retrieved.

The operational characteristics of the chamber were based on smoke dispersion tests, dust deposition tests and previous experience with the smaller chamber. The design characteristics proved to be satisfactory based on two tests of the chamber to date. Measurements made in the first test showed a mean deposition of 3.4×10^4 microorganisms per ribbon on 4 ribbons with a coefficient of variation of 27%. The second test resulted in a mean of 3.7×10^4 spores per ribbon on 8 ribbons with a coefficient of variation of 15%.

For heat resistance testing, 10 gm of dust consisting of equal amounts of sieve-processed vacuum cleaner dusts from Hangars AE, AO, and S, the O and C High Bay and the MSOB High Bay were aerosolized onto 32 sterile teflon ribbons as described above. The individual dusts were processed through a series of sieves in a dry state (Q.R. #32). The last sieve had a pore size of 43 μ and the particle size distributions of the resultant dusts were similar to the distributions found in natural airborne fallout (Q.R. #31). The ribbons were retrieved in sterile, numbered 1-quart Mason jars which were subsequently randomized before heating. Six jars were heated at each separate interval of 6, 12, 18 and 24 hr in a forced air oven in the laminar flow clean room. Temperature was maintained at 125 ± 1 C as determined at each interval by three control jars containing teflon ribbons and thermocouples. The comeup time of the system to 125 C was 40 min. At the beginning of each interval, two randomly selected jars were assayed in the following manner to determine the N_0 spore value. Five hundred ml of sterile .002% TW 80 solution were added to the jars and they were insonated in an ultrasonic bath for 6 min. The jars were then shaken vigorously by hand 25 times and re-insonated for 6 min. Twenty ml were removed from each jar, heat shocked (80 C, 15 min) and plated with TSA supplemented with 0.1% soluble starch and 0.2% yeast extract. Plates were incubated at 32 C for 72 hr. At the end of each heating interval, the six test jars were removed from the oven and were allowed to cool in a laminar flow clean bench for 15 min. Seven hundred ml of broth made according to the formula of TSA minus the agar and supplemented with 0.1% soluble starch and 0.2% yeast extract were added to each test jar in addition to two control jars containing sterile ribbons. Jars were incubated at 32 C for 28 days and were inspected at intervals for signs of growth.

Portions of the media from jars suspected of being positive were transferred to plates and tubes of fresh media for confirmation of growth.

Preliminary results have shown the following after 2 weeks of incubation:

Time at 125 C (hr)	N_0 (spores)	No. +/6	Control
6	3.7×10^4	1	0
12	3.8×10^4	2	0
18	3.4×10^4	0	0
24	4.0×10^4	0	0

Growth in the jars has been confirmed as Gram-positive, sporeforming rods. Further results will be reported next quarter.

2. Tests were performed to determine the comparative degree of heat resistance exhibited by naturally occurring spores in each of the sieve-processed vacuum cleaner dust samples described above. One gm of each dust sample was suspended in 10 ml of filter sterilized 95% ethanol and was used subsequently as an inoculum for heat testing by placing 0.1 ml amounts on 1/2" x 1/2" stainless steel strips. Replicates of ten strips were heated at 125 C for various intervals to obtain end point data for D_{125C} value calculations (Q.R. #26). The results are summarized in Table 1.

   The composite dust used in the teflon ribbon experiment has been assayed in a similar manner and results will be presented next quarter.

3. The NASA Standard Procedures for the Microbiological Examination of Space Hardware specify the use of a long-term slit sampler (Reyniers or equivalent) for sampling intramural air. However, Reyniers has not manufactured samplers for several years and no equivalent sampler has been available until recently when Unit Electric Control, Inc. (Box 3427, Orlando, Florida), marketed the M/G sampler. The M/G sampler has several attractive features not found on the Reyniers: (1) compact built-in vacuum pump, (2) built-in flow rate gauge and adjustment valve, (3) interchangeable drive motors, (4) built-in adjustable timer with shut-off switch, and (5) transparent, easily removed sample chamber.

   An M/G sampler was purchased for the purpose of comparing its performance with that of the Reyniers sampler. In calibrating the flow rate gauge on the sampler against a Fischer and Porter precision flowrator meter it was found that the maximum rate at which air could be sampled was 52 cfh, although the gauge on the sampler read approximately 60 cfh. This problem was discussed with the manufacturer and the sampler was returned to be refitted with a new pump. When the refitted sampler was returned it was again calibrated and found to sample a maximum of 53 cfh which indicated approximately 60 cfh on the sampler gauge.

Tests were conducted to determine what effect this limited sampling rate would have on the measurement of airborne microbial contamination. One Reyniers sampler drawing 60 cfh, one Reyniers sampler drawing 53 cfh, and the M/G sampler drawing 53 cfh were operated simultaneously in a room with well mixed air for a total of thirteen 30-minute sampling periods. The 60 cfh Reyniers detected a total of 1760 viable particles during this time for a mean concentration of 4.5 viable particles per true ft^3. The 53 cfh Reyniers detected 1415 viable particles for a mean concentration of 4.1 viable particles per true ft^3. The 53 cfh M/G detected a total of 695 viable particles for a mean concentration of 2.0 viable particles per true ft^3. If the 60 cfh indicated on the sampler gauge were used instead of the true rate of 53 cfh the detected concentration would be only 1.8 viable particles per ft^3. This means that using the M/G sampler, as purchased, would result in detecting levels of airborne microorganisms only 40 percent as high as those detected by the Reyniers sampler as we have used it.

The observation that when the Reyniers sampler and M/G sampler were both operated at 53 cfh the M/G detected only 49 percent (2.0 vs 4.1 viable particles per ft^3) as many airborne microorganisms as the Reyniers suggested a difference in sampling efficiency independent of airflow. It is possible that the knife-edge construction used in the Reyniers slit results in more efficient deposition of particles at lower flow rates than does the square-edge construction of the M/G slit. However, when the sampling rate of the M/G sampler was increased to a true 60 cfh through the use of a larger pump the M/G and Reyniers detected comparable concentrations of airborne microorganisms (1.7 and 1.6 viable particles per ft^3, respectively).

At this point it appears necessary to check the accuracy of the flow meters used in our laboratories with those used by the manufacturer of the M/G sampler. If our meters are correct it would seem that the manufacturer must increase the size of the pump in the M/G sampler as well as improve the accuracy of the flow gauge used on the sampler. If our meters were in error appropriate adjustments must be made along with a review of data which may be influenced by errors of the magnitude described here. In either event, further evaluation of the M/G sampler is needed before it can be accepted as an equivalent of the Reyniers.

4. NASA Standard Procedures for the Microbiological Examination of Space Hardware describe the rodac plate method and the swab-rinse method for the microbiological sampling of environmental surfaces. While the swab-rinse method is more widely used than the rodac method, both sample relatively small areas and consequently have certain limitations. One limitation is that many samples must be collected and processed to validly extrapolate sample levels to the larger surface being sampled. A second limitation is that results from many samples collected from surfaces with low levels of microbial contamination will be zero. The statistical interpretation of these values is made difficult by the fact that both methods are approximately 50% efficient.

A technique has been developed that overcomes these problems by taking a larger sample. The sample is collected by wiping the entire surface of interest with a sterile, premoistened, rayon cloth, commercially available as Handi Wipes (Colgate-Palmolive Co.). The microorganisms collected by the cloth are removed by rinsing and the rinse solution assayed.

Preliminary tests, in which known numbers of microorganisms were inoculated onto sterile sampling cloths, demonstrated the ability of this cloth to consistently release over 99% of the inoculum into the rinse solution during a five-minute agitation period. Agitation on a mechanical shaker for five minutes at a rate of 200 shakes per minute was compared with insonation for a two-minute period and equivalent removal results were obtained. Other tests ruled out the presence of inhibitory substances in the various colored cloths.

To test this sampling technique on smooth surfaces, 10 aluminum trays (11" x 16") were exposed to naturally occurring microbial fallout. Two 2" x 2" stainless steel strips were placed on each tray and were retrieved and assayed according to Standard Procedures to estimate the mean level of total microorganisms per square inch of tray surface. Each tray was wiped with a 7" x 8" sterile cloth moistened with approximately 7 ml of standard rinse solution. The cloth was used as one would use a cleaning cloth, turning and folding as necessary to clean the entire tray surface. A sterile, disposable vinyl medical glove was worn to prevent extraneous microbial contamination from reaching the cloth. Each cloth was placed in a 400 ml beaker containing 100 ml of rinse solution and the beaker was insonated for 2 minutes. Replicate plates of portions of the rinse solution were poured, incubated at 37 C and counted at 24 and 48 hours. The mean level of total microorganisms on the 10 trays was determined to be 19 per in^2. The mean recovery value for the 10 sampling cloths was 50% with a coefficient of variation of 24%. These values are comparable to those for the swab-rinse technique; however, the results may well be more valid for the wiping cloth technique because each sample in this case was based on an area 42 times that used for a single sample with the swab-rinse technique. Further work is planned to standardize and evaluate the wiping cloth technique for use on surfaces of space hardware.

The technique also has been used for skin sampling, particularly the microbiological assay of hands. In this procedure, the palms, backs and fingers of both hands were wiped with the same cloth, which was then placed in a 500 ml plastic jar containing 250 ml of sterile rinse solution. The jar was tightly sealed and then agitated for five minutes on a mechanical shaker.

The rinse solution may be assayed in a variety of ways to detect and enumerate specific microorganisms. In the scheme used in the development of this technique, four 50 ml portions were filtered through .45 μ membrane filters and the filters were placed on Trypticase Soy Agar and incubated

for four to six hr at 37 C. Two filters were then transferred to Vogel-Johnson agar and incubated for 48 hr at 37 C for detection and enumeration of Staphylococcus aureus. The remaining two filters were transferred to FC agar for detection and enumeration of fecal coliforms after 24 hr incubation at 44.5 C. Three 10 ml, three 1 ml and three .1 ml portions of the rinse solution were placed in tubes of asparagine broth and a MPN for Pseudomonas aeruginosa was determined after incubation for 48 hr at 37 C. Blood agar spin plates were inoculated with .5 ml at 10^0 and 10^{-1} dilutions of rinse solution, incubated at 37 C for 48 hr and examined for hemolytic streptococci. Total counts were determined from TSA pour plates incubated at 37 C for 48 hr.

In a series of experiments, a surface was uniformly inoculated with fecal coliforms, S. aureus and P. aeruginosa and subsequently handled by two individuals in a standardized manner. The hands of the individuals were sampled before and after touching the surface and, in two experiments, after hand washing. The results (Tables 2 and 3) showed that even with extremely low levels of contamination on the surface, fecal coliforms were detected on hands that had touched it. For both fecal coliforms and S. aureus, there appeared to be a direct relationship between the level on the surface and the level recovered from hands. It was also evident that hand washing did not consistently remove the acquired contaminants. In one experiment, this assay technique detected P. aeruginosa on hands of both individuals after touching a surface which had an average level of contamination of less than one microorganism per in^2.

The ability of this technique to detect and enumerate different types of microorganisms on a variety of surfaces suggests several areas of applicability in space science. For instance, by combining wiping cloth sampling with membrane filtration and culturing on selective media, a compact system for monitoring the types and levels of microorganisms on spacecraft surfaces as well as on the skin of astronauts could be developed for use in space.

5. A microbiological study was performed on the outbound automated Pioneer F spacecraft at Cape Kennedy, Florida prior to launch. A total of twenty-six (26) sites, each consisting of 4 square inches, were assayed. Quantitative results were submitted to the Planetary Quarantine Officer. Bacterial colonies were picked from TSA plates and three types of microorganisms were detected: Staphylococcus spp. Subgroup V, atypical Bacillus spp. and an actinomycete.

6. The study to determine the thermal resistance of naturally occurring airborne spores (Q.R. #35 and #36) was continued. Sixteen aluminum trays (7" wide x 74" long x 40" high) containing two 3" x 72" ribbons of teflon (5 mil FEP Dupont) were located in the Low Bay area of the Manned Spacecraft Operations Building (MSOB). This area is used for the assembly of the Apollo spacecraft. The teflon ribbons were cleaned in the same manner as SS strips, and new ribbons were preconditioned by two exposures

to dry heat for 3 hr each at 175 C prior to a third heating interval for sterilization. Ribbons were exposed to intramural fallout for 7 days, and at this point, each ribbon was rolled up and aseptically (sterile gloves and masks) placed in sterile Mason jars. Control and test ribbons were selected randomly and eight of the 32 ribbons used for N_0 determinations. For each test, 6 test jars and 3 control jars (each control containing an uninoculated ribbon and a thermocouple) were heated at 125 C ± 1 C. The relative humidity of the assembly area was monitored constantly and the relative humidity of the laboratory testing area was monitored during the heating intervals. The test assay medium was TSA broth (same formula as TSA except without agar) plus 0.2% yeast extract and 0.1% soluble starch; TSA plus yeast extract and starch was used for determining total and spore counts on control ribbons. Each control ribbon was rolled up and placed in a sterile jar, 400 ml of sterile rinse solution (.002% Tween 80) was added and the jars were insonated for 6 minutes, removed, manually shaken 25 times and then insonated again for 6 minutes. Five ml portions were plated in duplicate for total counts, and duplicate 50 ml portions were heat shocked (80 C, 25 min) and plated for spore counts. Plates were incubated at 32 C and colonies counted after 2, 3 and 7 days. After the jars containing the test ribbons were heated, 400 ml of sterile broth was added aseptically and the jars were incubated at 32 C and observed for the presence of visible growth at 7 days and weekly up to 28 days. Eight jars containing teflon ribbons and 400 ml of broth were included as sterility controls in each run of 24 test ribbons.

Four experiments were run in the "old area" of the MSOB prior to moving the fallout tables (Q.R. #36). The summarized data from these four experiments are shown in the first part of Table 4. The heating times used in all four experiments were 3, 6, 9 and 12 hours. The second part of Table 4 summarizes the data for the experiments run in the "new area." Thus far, fourteen experiments have been run in the "new area" with the first five using the heating times 1, 2, 3 and 4 hours and the remaining nine experiments using 2, 4, 6 and 8 hours. The N_0 values shown are the average of eight control strips per experiment. The N_0 values for total counts and bacterial spores in the "new area" are generally lower than the "old area" and they tend to show more variation. Experiments are continuing in the "new area" using the heating times 2, 4, 6 and 8 hours.

A summary of all the ribbon experiments to date according to heating time and location is shown in Table 5. The N_0 values shown are the average of all the control strips at that heating interval (two per experiment). The longest heating period yielding survivors thus far is six hours. D_{125C} values were calculated using the FN-MPN technique of Pflug and Schmidt. D_{125C} values ranged from 25 to 103 minutes depending on the length of exposure to heat. The 3 and 6 hour heating intervals showed D_{125C} values that are relatively close in both areas.

7. Demonstration of a consistent and predictable relationship between the concentration of total particles and viable particles suspended in ambient air would dramatically reduce laboratory work and permit continuous

monitoring of airborne microbial contamination. Studies were conducted in a class 100,000 clean room in the MSOB as well as in the Spacecraft Bioassay Laboratory to determine whether such a relationship exists. In both areas, air was sampled simultaneously at contiguous sites with a Royco particle counter and an Andersen sampler to measure total and viable particle concentrations respectively. The Royco counter was adjusted to count particles within size ranges matched to those of the six stages of the Andersen sampler as shown in Table 6.

Although environmental controls and the nature of the activities in the two test areas were dissimilar, Table 7 shows that the distribution of viable particles by particle size was similar. The particle size distribution for all particles, as measured by the Royco, is shown in Figure 2 and a general similarity between the two areas is evident with higher total particulate levels existing in the Spacecraft Bioassay Laboratory than in the MSOB. Figure 3 graphically expresses viable particle concentrations as a percentage of total particle concentrations for each particle size range. In analyzing these data, it was found that over 99% of the total particles in these areas were less than 1 μ in size; however, less than 1 in 10,000 particles of this size was a viable particle. At the other end of the particle size scale, it was found that less than 1/10 of 1% of the total particles were greater than 5.5 μ in size; but, approximately 45% of the particles in this range were viable particles. Because viable particles make up a sizeable portion of the total particles over 5.5 μ, it was felt that if a relationship between total particles and viable particles existed, it would be most evident among particles in this size range. A regression analysis of the combined data from both areas relating total particles over 5.5 μ to viable particles over 5.5 μ was performed and a gross correlation was detected. The points used in this analysis are shown in Figure 4. While this correlation suggested that as the concentration of total particles increased, the concentration of viable particles increased, regression analysis of the data from each area individually showed no correlation. The absence of a consistent relationship led to the conclusion that total particle concentrations cannot be used to estimate viable particle concentrations.

8. Identification of Bacillus species using biochemical methods is not a simple matter. As compared to medically significant groups of microorganisms (Staphylococcus spp., for instance), relatively little is known about the physiological stabilities of Bacillus spp. as they are isolated from their natural environments and cultured in the laboratory. Therefore, studies have been initiated to examine the possibility of shifts in biochemical reaction patterns during storage and subculture of environmental isolates.

   In order to perform a preliminary study, five environmental Bacillus isolates were subjected to three types of handling prior to identification by the scheme used with isolates from Apollo spacecraft:

   a. Two sequential subcultures on brain heart infusion (BHI) as is routine for Apollo isolates.

b. Two transfers as in a. above and storage at 4 C for 5 weeks.
c. Ten sequential subcultures on BHI, twice weekly for 5 weeks.

Results of biochemical tests run in triplicate are shown in Table 8. The ability to grow at 50 C was gained by each isolate after both storage and ten sequential subcultures. There were also variations in the abilities to utilize mannitol and citrate and to reduce nitrate to nitrite. In the majority of instances, one or more of the variations changed the subsequent identification of an isolate. With one isolate (no. 4), two distinct colonial types varying additionally in ability to utilize citrate were detected after ten subcultures.

Another group of fresh environmental isolates is currently being tested to confirm the observation of critical variations in biochemical characters due to subculture and storage in the laboratory. The accuracy of the Apollo identification scheme also is being evaluated using a series of standard Bacillus, spp. cultures supplied by Dr. Ruth Gordon of Rutgers University. Since the Apollo identification scheme is based primarily on the 1952 USDA Monograph No. 16 by Smith, Gordon and Clark, Dr. Gordon's new USDA monograph (in the process of being published) may necessitate changes in the Apollo scheme.

TABLE 1. D_{125C} VALUES OF SIEVE-PROCESSED VACUUM CLEANER DUST CALCULATED FROM FN-MPN DATA

Source	N_0 (spores)	D-value (hr)	Last hourly interval with positives
Hangar AE	3.1×10^3	1.2	4
O and C High Bay	3.7×10^2	1.0	4
MSOB High Bay	4.0×10^3	2.4	13
Hangar AO	1.4×10^3	2.5	13
Hangar S	1.4×10^3	0.9	4

TABLE 2. DETECTION OF FECAL COLIFORMS ON HANDS AFTER TOUCHING CONTAMINATED SURFACE

Experiment	Subject	Mean No. Fecal Coliforms Per in^2 on Surface	No. Fecal Coliforms Recovered from Hands		
			Before Touching	After Touching	After Washing
1	A	1	0	5	--
1	B	1	0	3	--
2	A	16	0	10	--
2	B	16	0	25	--
3	A	320	0	2200	10
3	B	320	0	300	0

TABLE 3. DETECTION OF S. AUREUS ON HANDS AFTER TOUCHING CONTAMINATED SURFACE

Experiment	Subject	Mean No. S. aureus Per in^2 on Surface	No. of S. aureus Recovered from Hands		
			Before Touching	After Touching	After Washing
1	A	180	--	500	--
1	B	180	0	500	--
2	A	770	300	7000	110
2	B	770	0	4000	140

TABLE 4. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY

EXPERIMENT NUMBER		N_0 TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
1	Old Area	5.4×10^3	2.4×10^2	2.4×10^2	3 hr. - 3/6	B. circulans-2, Atypical Bacillus
					6 hr. - 0/6	
					9 hr. - 0/6	
					12 hr. - 0/6	
2	"	4.0×10^3	1.2×10^2	8.0×10^1	3 hr. - 0/6	
					6 hr. - 0/6	
					9 hr. - 0/6	
					12 hr. - 0/6	
3	"	3.1×10^3	1.4×10^2	1.6×10^2	3 hr. - 0/6	
					6 hr. - 0/6	
					9 hr. - 0/6	
					12 hr. - 0/6	
4	"	4.1×10^3	4.3×10^2	1.6×10^2	3 hr. - 0/6	
					6 hr. - 1/6	Atypical Bacillus
					9 hr. - 0/6	
					12 hr. - 0/6	
5	New Area	4.9×10^3	2.7×10^2	3.6×10^3	1 hr. - 5/6	B. sphaericus-2, B. lentus, B. firmus, Atypical Bacillus
					2 hr. - 3/6	B. cereus, B. firmus, Atypical Bacillus
					3 hr. - 1/6	B. polymyxa
					4 hr. - 1/6	B. subtilis
6	"	3.8×10^3	2.6×10^2	2.6×10^3	1 hr. - 4/6	B. lentus-2, B. subtilis, Atypical Bacillus
					2 hr. - 3/6	B. polymyxa, B. pantothenticus, Atypical Bacillus
					3 hr. - 0/6	
					4 hr. - 2/6	B. lentus-2

TABLE 4. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY (Continued)

EXPERIMENT NUMBER		N_0 TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
7	New Area	1.4×10^3	1.4×10^2	4.8×10^2	1 hr. - 1/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	B. sphaericus
8	"	4.0×10^2	4.0×10^1	2.4×10^2	1 hr. - 0/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	
9	"	4.8×10^2	5.6×10^1	8.0×10^1	1 hr. - 0/6 2 hr. - 0/6 3 hr. - 0/6 4 hr. - 0/6	
10	"	5.6×10^2	1.5×10^2	1.6×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
11	"	1.3×10^3	2.1×10^2	4.0×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
12	"	6.4×10^2	8.8×10^1	2.4×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	
13	"	1.4×10^3	1.4×10^2	9.6×10^2	2 hr. - 0/6 4 hr. - 0/6 6 hr. - 0/6 8 hr. - 0/6	

TABLE 4. THERMAL RESISTANCE OF NATURALLY OCCURRING AIRBORNE BACTERIAL SPORES COLLECTED ON EXPOSED TEFLON RIBBONS - CAPE KENNEDY (Continued)

EXPERIMENT NUMBER		N_0 TOTAL COUNT	SPORES	MOLDS	SURVIVORS	IDENTIFICATION
14	New Area	1.1×10^3	1.3×10^2	2.4×10^2	2 hr. - 3/6	B. circulans, Actinomycete, In Process
					4 hr. - 1/6	Actinomycete
					6 hr. - 0/6	
					8 hr. - 0/6	
15	"	4.0×10^2	5.6×10^1	1.6×10^2	2 hr. - 1/6	B. sphaericus
					4 hr. - 1/6	In Process
					6 hr. - 0/6	
					8 hr. - 0/6	
*16	"	4.0×10^2	7.2×10^1	1.6×10^2	2 hr. - 0/6	
					4 hr. - 0/6	
					6 hr. - 0/6	
					8 hr. - 0/6	
*17	"	6.4×10^2	6.4×10^1	3.2×10^2	2 hr. - 0/6	
					4 hr. - 0/6	
					6 hr. - 0/6	
					8 hr. - 0/6	
*18	"	1.5×10^3	2.1×10^2	4.0×10^2	2 hr. - 1/6	In Process
					4 hr. - 0/6	
					6 hr. - 2/6	In Process-2
					8 hr. - 0/6	

*Experiments in which incubation is in progress.

TABLE 5. SUMMATION OF RESULTS FROM TEFLON RIBBON EXPERIMENTS - CAPE KENNEDY

HEATING TIME	NO. OF EXPERIMENTS	N_0 VIABLE	N_0 SPORES	N_0 MOLDS	NO. SURVIVORS PER TOTAL NO. FLASKS	D_{125C} VALUE	IDENTIFICATION
MSOB - NEW AREA							
1 hr.	5	2.7×10^3	1.0×10^2	1.8×10^3	10/30	25 min.	B. lentus-3, B. sphaericus-3, B. firmus, B. subtilis, Atypical Bacillus-2
2 hr.	14	1.2×10^3	1.4×10^2	6.4×10^2	12/84*	41 min.	B. sphaericus, B. cereus, B. firmus, B. polymyxa, B. pantothenticus, B. circulans, Atypical Bacillus Actinomycete-2, Lost in Process In Process-2
3 hr.	5	1.8×10^3	2.1×10^2	1.2×10^3	1/30	47 min.	B. polymyxa
4 hr.	14	1.4×10^3	1.4×10^2	7.2×10^2	5/84*	71 min.	B. subtilis, B. lentus-2, Actinomycete, In Process
6 hr.	9	7.2×10^2	1.2×10^2	2.4×10^2	2/54*	103 min.	In Process-2
8 hr.	9	1.1×10^3	1.0×10^2	4.0×10^2	0/54*	--	----
MSOB - OLD AREA							
3 hr.	4	3.6×10^3	2.2×10^2	8.0×10^1	3/24	56 min.	B. circulans-2, Atypical Bacillus
6 hr.	4	4.3×10^3	2.2×10^2	1.6×10^2	1/24	97 min.	Atypical Bacillus
9 hr.	4	4.0×10^3	2.0×10^2	1.6×10^2	0/24	--	----
12 hr.	4	4.6×10^3	2.6×10^2	2.4×10^2	0/24	--	----

*Includes experiments in which incubation is in progress.

TABLE 6. PARTICLE SIZE RANGES FOR DATA COLLECTION WITH THE ROYCO PARTICLE COUNTER AND ANDERSEN SAMPLER

	PARTICLE SIZE IN MICRONS	
Stage	Andersen	Royco
1	>9.2	>9.5
2	5.5-9.2	5.5-9.5
3	3.3-5.5	3.5-5.5
4	2.0-3.3	1.5-3.5
5	1.0-2.0	.95-1.5
6	<1.0	<.1-.95

TABLE 7. PARTICLE SIZE DISTRIBUTION OF VIABLE PARTICLES IN TWO TEST AREAS

Particle Size Microns	Percentage of All Viable Particles in Each Size Range	
	Spacecraft Bioassay Laboratory	MSOB
> 9.2	42	40
5.5-9.2	24	29
3.3-5.5	9	5
2.0-3.3	13	10
1.0-2.0	10	12
< 1.0	2	4

TABLE 8. A COMPARISON OF BIOCHEMICAL REACTIONS OF FIVE *BACILLUS* ISOLATES AFTER THREE TYPES OF HANDLING PRIOR TO IDENTIFICATION

ISOLATE NO.	TREATMENT	TESTS										IDENTIFICATION
		GROWTH 50 C	ANAEROBIC GLUCOSE	ANAEROBIC NITRATE	MANNITOL	STARCH	GELATIN	CITRATE	VP	NITRATE	INDOL	
	Transferred 2X	-	+	+	+	+	+	-	-	+	-	*B. circulans*
1	Stored 5 weeks	+	+	+	-	+	+	-	-	+	-	*B. circulans*
	Transferred 10X	+	+	+	+	+	+	-	-	+	-	*B. circulans*
	Transferred 2X	-	+	+	+	+	+	-	+	+	-	*B. polymyxa*
2	Stored 5 weeks	+	+	+	-	+	+	-	+	+	-	*B. cereus*
	Transferred 10X	+	+	+	-	+	+	-	+	+	-	*B. cereus*
	Transferred 2X	-	+	+	+	+	+	-	+	+	-	*B. polymyxa*
3	Stored 5 weeks	+	+	+	-	+	+	-	+	+	-	*B. cereus*
	Transferred 10X	+	+	+	+	+	+	-	+	+	-	Atypical *Bacillus*
	Transferred 2X	-	+	-	+	+	+	-	-	+	-	*B. circulans*
4	Stored 5 weeks	+	+	-	+	+	+	+	-	-	-	Atypical *Bacillus*
	*Transferred 10X a	+	+	-	+	+	+	-	-	-	-	*B. circulans*
	*Transferred 10X b	+	+	-	+	+	+	+	-	-	-	Atypical *Bacillus*
	Transferred 2X	-	+	-	+	-	+	-	-	-	-	*B. pulvifaciens*
5	Stored 5 weeks	+	+	-	-	-	+	-	-	-	-	*B. badius*
	Transferred 10X	+	+	-	-	-	+	-	-	-	-	*B. badius*

*Two distinct colonial types

Transferred 2X -- as done for Apollo isolates
Stored 5 weeks -- on BHI slants at 4 C
Transferred 10X -- twice weekly for 5 weeks

All biochemical tests were run in triplicate

FIGURE 1. CUTAWAY VIEW OF DUST AEROSOLIZATION CHAMBER

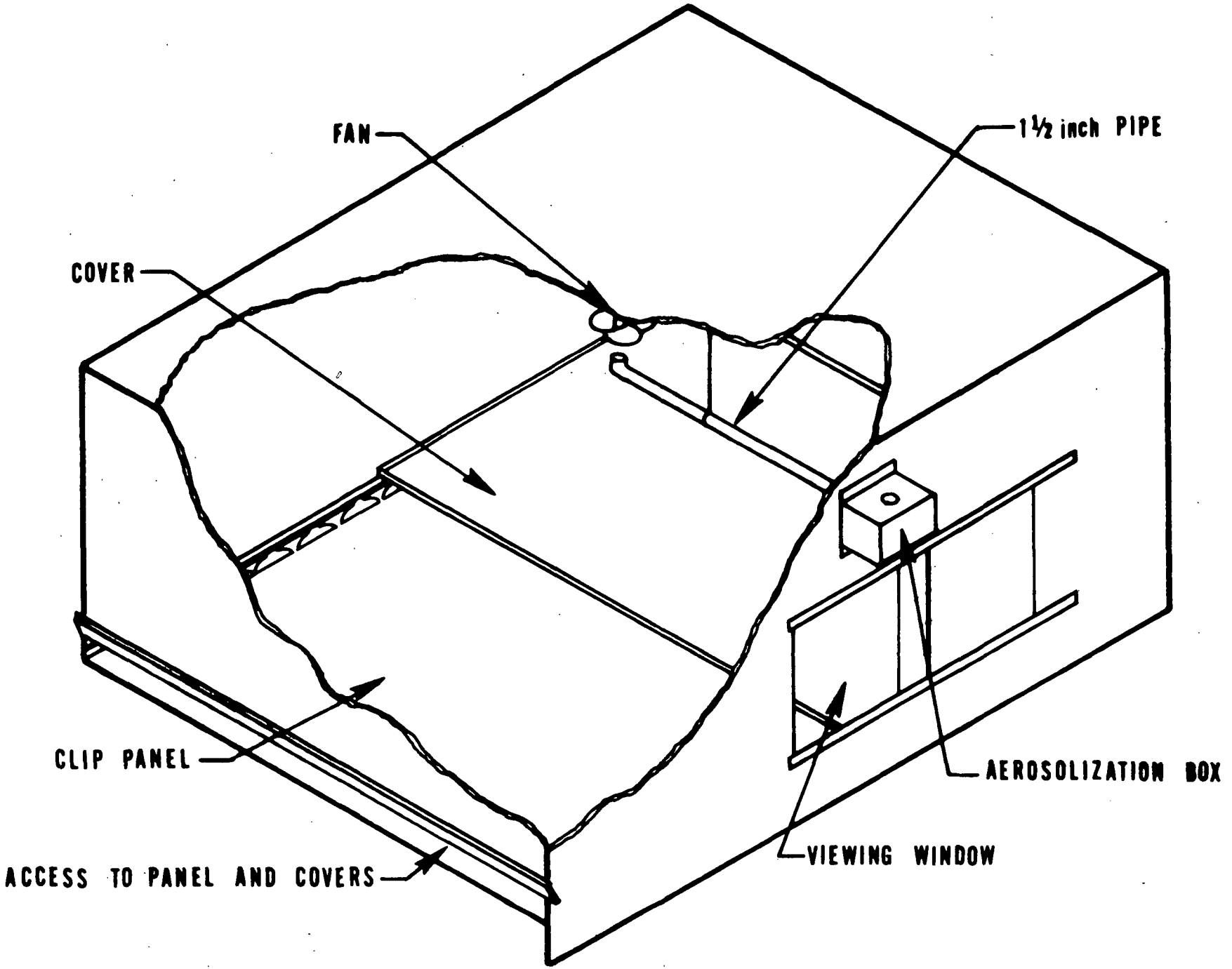

FIGURE 2. PARTICLE SIZE DISTRIBUTION FOR TOTAL PARTICLES IN TWO TEST AREAS

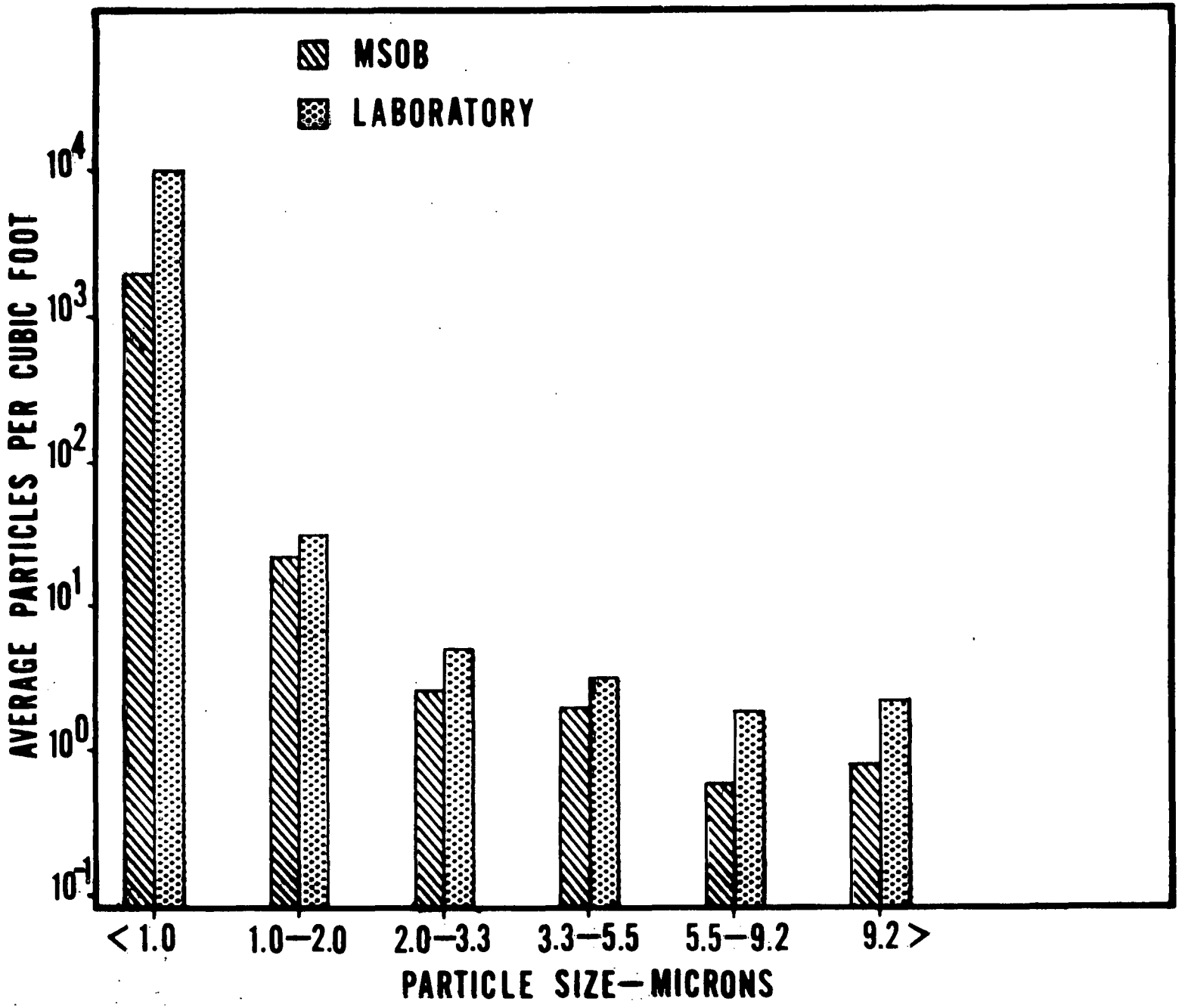

FIGURE 3. VIABLE PARTICLE CONCENTRATIONS EXPRESSED AS PERCENTAGES OF TOTAL PARTICLE CONCENTRATIONS

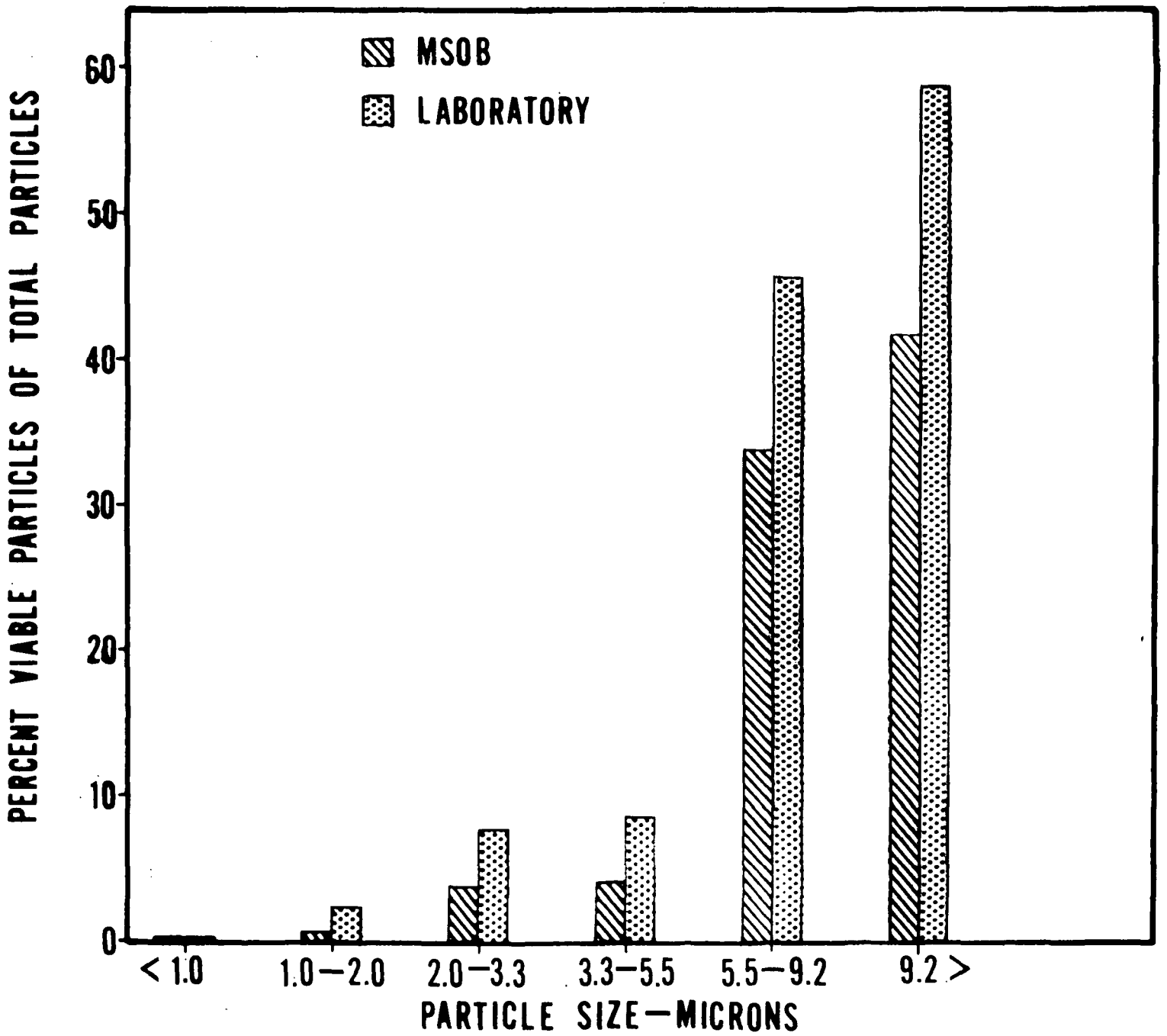

FIGURE 4. DATA POINTS USED IN REGRESSION ANALYSIS OF VIABLE PARTICLE CONCENTRATIONS AND TOTAL PARTICLE CONCENTRATIONS